PB86-201472

Virus Adsorption to Mineral Surfaces Is Reduced by Microbial Overgrowth and Organic Coatings

New York State Dept. of Health, Albany

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

1985

U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA/600/J-85/400	2.	3. RECIPIE PB86-201472
4. TITLE AND SUBTITLE Virus Adsorption to Mineral Surfaces Is Reduced by Microbial Overgrowth and Organic Coatings (Journal Version)		5. REPORT DATE 1985 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) G. Wolfgang Fuhs, Min Chen, Lawrence S. Sturman, and Rebecca S. Moore		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS New York State Department of Health Center for Laboratories and Research Empire State Plaza Albany, NY 12201		10. PROGRAM ELEMENT NO. CAYB1A 11. CONTRACT/GRANT NO. R804743
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES
Project Officer: E.W. Akin, Chief, MB, TMD, HERL (CI)
Microbial Ecology 11:25-39, MARCH 1985

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified 20. SECURITY CLASS (This page) Unclassified	21. [illegible] PAGES 22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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JOURNAL ARTICLE

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Microb Ecol (1985) 11:25–39

MICROBIAL ECOLOGY
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Virus Adsorption to Mineral Surfaces Is Reduced by Microbial Overgrowth and Organic Coatings

G. Wolfgang Fuhs, Min Chen, Lawrence S. Sturman, and Rebecca S. Moore*

New York State Department of Health, Wadsworth Center for Laboratories and Research, Empire State Plaza, Albany, New York 12201, USA

Abstract. In experiments with strains of poliovirus, reovirus, echovirus, and Coxsackievirus, overgrowth with exopolymer-forming bacteria reduced virus adsorption to mineral surfaces. Adsorption was improved when organic materials adsorbed to minerals were removed by low-temperature ashing. In a soil series, virus adsorption increased with soil depth. This paralleled a decrease in organic content, but differences in charge development of the soil particles may also be involved. Prolonged (40-year) irrigation with treated sewage effluents, leading to a buildup of organic coatings, also decreased adsorption. However, saturation of the virus-binding capacity of the soil as a result of continuous exposure to virus-containing effluents was not apparent.

Introduction

The adsorption of viruses to soils occurs in the recharge of natural aquifers with treated wastewater effluents, in septic tank leachfields, and around sanitary landfills. It is not only of practical interest but also provides examples of interactions of virus particles with other organic colloids and mineral surfaces. Organic matter appears to affect the adsorption of virus particles in that (a) dissolved and colloidal organic matter competes with virus for adsorption sites on the mineral surfaces [e.g., 2, 3, 12, 15] and (b) adsorption to organic surfaces is poorer than adsorption to many minerals [17]. Whereas other authors found that, with certain unexplained exceptions, the removal of virus from suspension increases with organic content of the soil [4, 6], our studies with 5 viruses and over 40 minerals and soils show a strong inverse relationship between virus adsorption and organic carbon content [13, 14].

In this article we report on several experiments which were carried out as part of our larger study and which essentially deal with the effect on virus adsorption of organic coatings which are produced on mineral surfaces by microbial colonization. Such overgrowth occurs regularly in topsoils even without additions of sewage and is additional to the adsorption of organic matter

* *Present address:* Rollins Animal Disease Diagnostic Laboratory, North Carolina Department of Agriculture, Raleigh, North Carolina, USA.

(polar and nonpolar lipids, proteins, humic matter) to mineral surfaces exposed to polluted and unpolluted natural waters [16].

Materials and Methods

Viruses and Cell Lines

Poliovirus type 2 (vaccine strain P712-ch-2ab), reovirus type 3 (Dearing strain), echovirus type 7, and Coxsackievirus B5 were produced and assayed in monolayer cell cultures of the following types: HeLa, L2, Vero, and FL amnion, respectively. Echovirus type 1 was produced in Vero cells and assayed in FL amnion cells.

Virus Production and Purification

Production and purification of radiolabeled poliovirus 2 and reovirus 3 have been described previously [13, 14]. Cell cultures infected with echovirus types 1 and 7 or Coxsackievirus at multiplicities of 5–25, 10–20, or 1, respectively, were labeled with [^{35}S] methionine, 4.0–7.5 μCi/ml (>400 μCi/mM) at 2–6 hours after infection in Dulbecco's modified Eagle's medium lacking methionine (echovirus 7), containing $\frac{1}{20}$th the usual amount (Coxsackievirus), or the usual amount of methionine (echovirus 1), supplemented with 5% fetal bovine serum. Cell-associated virus was harvested at 24 hours. Echovirus 1 was purified by Genesolv-D extraction and isopycnic sedimentation in CsCl similar to reovirus [14]. Echovirus 7 and Coxsackievirus were purified at 4°–10°C as follows: The cells were pelleted by centrifugation in a GSA rotor at 4,000 *g* for 20 min in a Sorvall RC2 or RC5 centrifuge, and then the pellet was resuspended in Dulbecco's PBS with (Coxsackievirus) or without (echovirus 7) Ca^{2+} and Mg^{2+}(PBS_{def}), treated with 0.5% Nonidet P40 (NP40, Shell) and disrupted in a Dounce homogenizer. The lysate was clarified in an SS34 rotor at 1,500 *g* or 7,700 *g* for 10 min and the cell-associated virus was pelleted at 130,000 *g* for 2.5 hours in a Beckman L3-50 or L5-65B ultracentrifuge. This pellet was resuspended in PBS_{def} containing 0.5% NP40 using a Duall-style teflon tissue grinder (Kontes K-88538).

Coxsackievirus was purified by isopycnic sedimentation three times in preformed cesium chloride gradients and dialyzed against PBS_{def} after each sedimentation. Resuspended echovirus 7 was incubated overnight and rehomogenized with 0.5% NP40. The resuspended pellets were clarified by centrifugation at 7,000 *g* for 30 min and the virus in the supernatant was purified by isopycnic sedimentation on preformed cesium chloride gradients similarly to Coxsackievirus.

Virus Assays

Measurement of poliovirus and reovirus infectivity by plaque titration has been described [13, 14]. For the other viruses, the overlay medium for plaque assay contained Eagle's minimal essential medium supplemented with 5% fetal bovine serum and 0.8% Noble agar (echovirus 1 and 7) or 0.75% agarose (Coxsackievirus). Radioactivity was measured as described previously [13].

Substrates

A number of minerals and soils were used as substrates for virus adsorption. Ottawa sand was obtained from VWR Scientific, Rochester, N.Y., as no. 20117. Riverhead sandy loam and Texas Acuff loam were gifts from Dr. T. J. Tofflemire, New York State Department of Environmental Conservation, and Dr. T. D. Hinesly, Texas Christian University, Lubbock, Texas, respectively.

Table 1. Influence of low-temperature ashing and ignition of various substrates on their interaction with radiolabeled poliovirus

Substrate	Pre-treatment	Surface area (BET) (m^2/g)	Total carbon (%)	Organic carbon (%)	pH (synthet. freshw.)	PDADMA adsorbed (mg/g)	Removal of poliovirus[a] Infectivity (Logs)	Removal of poliovirus[a] Radioactivity (Logs)
Ottawa sand	None	0.01	0.15	≤0.15[c]	7.10	ND[b]	0.28	0.01
	Ashed	0.01	0.00	0.00	6.30	ND	0.84	0.43
Kaolinite	None	14.00	0.05	0.02	5.10	0.14	2.00	1.05
	Ashed	13.00	0.06	0.00	5.40	0.12	2.70	1.05
	Ignited	13.00	0.00		5.20	0.11	2.52	2.40
Genesee silt	None	9.20	0.19	0.15	7.50	0.24	2.70	1.89
	Ashed	7.30	0.20	≤0.20[c]	6.40	0.18	0.51	0.96
	Ignited	8.80	0.02	≤0.02[c]	6.70	0.11	0.36	0.68
Riverhead sandy loam, C-1	None	3.30	ND[b]		4.70	0.02	1.22	0.96
	Ashed	2.30	0.01	≤0.01[c]	6.10	0.00	1.70	0.96
	Ignited	2.50	0.01	≤0.01[c]	6.70	0.04	.96	1.05
Genesee river sed. no. 88	None	ND[b]	1.70	≤1.70[c]	5.90	ND[b]	0.00	0.19
	Ashed	ND	0.55	0.35	6.40	ND	1.22	0.89
	Ignited	ND	0.14	0.01	7.80	ND	0.15	1.19
Genesee river sed. no. 89	None	ND	1.50		7.00	ND	0.85	0.59
	Ashed	ND	0.58	0.31	7.00	ND	1.40	0.96
	Ignited	ND	0.22	0.01	8.40	ND	0.54	0.55

[a] Relative to substrate-free control
[b] Not determined
[c] Not determined separately, see previous column

Beach sand, Arizona montmorillonite, Genesee silt, muck, and magnetite sand were all obtained from Ward's Scientific Establishment, Rochester, N.Y. A brief description of each substrate is given below. Soil classifications are those by the US Department of Agriculture, Soil Conservation Service [21]. Classifications are provisional and can be confirmed only by a site visit.

Ottawa sand. This was the reference material for most experiments. It is 20–30 mesh and meets ASTM C190 specifications. Although not characterized extensively, analyses indicated that it was essentially inert, pure silica sand with only a small amount of surface impurity. It had the smallest surface area of all the substrates chosen (Table 1).

Beach Sand (New York, Lake Ontario). Predominantly coarse silica sand. Contains a calcareous component (about 4.5% CO_3).

Montmorillonite (Arizona) (American Petroleum Institute mixed layer aggregate no. 31). $(Mg,Al)_2Si_4O_{10}(OH)_2 \cdot nH_2O$. Montmorillonite is a highly ordered 2:1 layer clay mineral. Our sample was a relatively pure mineral. It had a low isoelectric point (below 3) as well as a strong affinity for the divalent cations in synthetic freshwater (see below). It was also a strong buffer and maintained suspensions at pH 8.5–9.0.

Genesee Silt (Industry, New York). A silt loam classified as Inceptisol, Fluventic Eutrochepts, fine-loamy, mixed, closely related to Hamlin Series. The upper layer (15–30 cm) had an organic carbon content of 1.65%. The lower layer (below 45–50 cm) contained more than 80% silt and less than 12% clay and contained only 0.15% organic carbon.

Table 2. Soil series: physical and chemical characteristics

Substrate (no., type)	Depth (cm)	Surface area[a] (BET) (m^2/g)	Surface area[a] (porosimetry) (m^2/g)	pH, synth. freshw.[b]	Organic carbon (%)	Wt. loss LTA[c] (%)	Wt. loss (Ignition) (%)	PDADMA adsorption[d] (mg/m^2)	Fe (H_2O_2-HNO_3 Extraction) (mg/g)	Al (H_2O_2-HNO_3 Extraction) (mg/g)	Ca (H_2O_2-HNO_3 Extraction) (mg/g)
Genesee silt loam											
24	15–30	4.2	0.87	8.1	1.65	3.36	5.56	1.10	19.0	11.0	1.75
29	30–60	11	0.81	8.0	0.15	0.95	2.14	0.17	25.0	12.5	0.525
Riverhead sandy loam											
52 (B21)	2–12	5.3	0.88	5.3	1.0	2.2	3.3	0.18	11.5	8.55	0.025
53 (B22)	12–40	7.3	0.82	5.5	0.45	1.28	1.87	0.078	6.0	7.95	0.05
54 (B31)	40–60	6.2	0.92	5.6	0.25	1.11	1.71	0.071	6.0	5.2	0.056
55 (B32)	60–90	3.1	0.68	6.3	0.20	0.47	0.81	0.084	4.4	1.55	0.056
56a (III, C1)	90–150	3.3	0.25	6.9	0.20	0.39	0.71	0.043	3.5	1.25	0.36
56 (III, C2)	150–180	1.25	0.15	7.4	0.10	0.04	0.21	0.10	1.7	4.5	0.09
Acuff loam, irrigation field											
101	0–15	6.0	0.25	6.9	1.65	1.24	2.30	0.35	6.3	9.1	0.456
102	15–30	10.2	0.18	7.2	0.60	0.86	1.66	0.22	6.95	9.55	0.425
103	30–60	18	0.28	7.5	0.35	0.70	1.91	0.16	9.95	14.5	0.42
Acuff loam, control field											
104	0–15	10.4	0.14	7.9	0.45	0.83	1.53	0.23	8.0	11.5	0.385
105	15–30	13	0.21	8.0	0.40	0.85	1.58	0.19	8.9	13.5	0.435
106	30–60	22	0.22	8.1	0.45	1.15	2.19	0.14	13.5	19	0.605

[a] Brunauer-Emmett-Teller (N_2 adsorption) and Hg intrusion (porosimetry) methods respectively; pressure during Hg intrusion was sufficient to fill pores of 30-nm diameter

[b] pH value for 0.5 g substrate in 2.0 ml synthetic freshwater

[c] Low-temperature ashing in an oxygen plasma

[d] Polydiallyl dimethylammonium chloride, per m^2 BET surface

Muck. A histosol composed of finely divided, well-decomposed organic matter intermixed with a high percentage of mineral matter, usually silt, and found in some poorly drained areas. This muck contained up to 50% organic matter. It had a very strong affinity for cationic polyelectrolyte [13], indicating substantial negative charge.

Magnetite Sand. This sand was about 65% magnetic, i.e., it was primarily magnetite, Fe_3O_4. About 80% of the remainder of the sample was also oxides, including hematite (Fe_2O_3) and ilmenite ($FeTiO_3$). The remainder was made up of feldspars and other minerals.

Riverhead Sandy Loam. Inceptisol, Typic Dystrochrept, coarse-loamy, mixed, mesic. This is an acid outwash soil typical of Long Island, New York; it was collected at Brookhaven National Laboratories, Upton, Long Island, New York. The subhorizons span the soil profile through the litter and organic accumulations, the topsoils, into the subsoils. With increasing depth the soil becomes less acidic and increasingly sandy. For those soils, the exact locations are recorded. Chemical, physical, and mineralogical analyses are also available [20].

Acuff Loam, Lubbock, Texas. Soils were made available to us from 2 sites. One of these sites had been irrigated with secondary-treated (trickling filter) sewage effluent for 40 years (Gray Farm), while the soils from the other site, 200 ft distant (Lubbock Compress Company land), had not. Samples from 3 horizons of each were obtained, not necessarily from identical sites. They were classified as Aridic Palenstoll, fine loamy, mixed, thermic. The soils are all similar in texture. They are weakly calcareous and have moderate ion exchange capacities. The soils exposed to effluent were neutral while those which were untreated were basic. In addition, there was more organic matter in the soils irrigated with effluent; the acidity of the resulting humic content may have counterbalanced the natural alkalinity of the soil. Results of these analyses are shown in Tables 1 and 2; other data are available from the study of Hinesly et al. [10].

Substrate Preparation and Characterization. This was done as described previously by Moore et al. [13]. The materials obtained from Ward's Scientific Establishment were ground in a tungsten–carbide ring mill, and the Riverhead and Acuff soils were kept at 4°C and ground lightly with mortar and pestle. For all substrates, the fraction passing a 2-mm sieve was used. The most important chemical properties of these substrates have been reported previously [13]. The properties of the Acuff loams are presented in Table 2. Measurements of surface area were by the nitrogen adsorption (Brunauer-Emmett-Teller, BET) technique and by mercury porosimetry (performed under contract with USEPA by the Carborundum Company, Niagara Falls, N.Y.). Mercury intrusion was under pressure sufficient to fill pores with an average hydrated diameter of a picornavirus (30 nm).

Suspending Solutions

An artificial freshwater medium contained, in mg liter^{-1}: KCl, 8; $MgSO_4 \cdot 7H_2O$, 92; $CaCl_2 \cdot 2H_2O$, 147; and $NaHCO_3$, 105; in distilled water filtered through a membrane filter (Millipore Corp., Bedford, Mass.). The pH was adjusted to 7.0 with 0.2 N NaOH, the solution was filter-sterilized and stored at 4°C in borosilicate-glass or plastic bottles. The pH was readjusted to 7.0 before use. The rationale for selecting this solution was presented previously [13]. The solution contains, in millequivalents per liter: sodium, 1.25; potassium, 0.10; magnesium, 0.75; calcium, 2.00; chloride, 2.10; sulfate, 0.75; and bicarbonate, 1.25.

For experiments with the Acuff soils, an arid-region synthetic freshwater was developed based on an analysis of secondary effluent from the Southeast Reclamation Plant at Lubbock, Texas [10]. The medium contained, in millequivalents per liter, $NaHCO_3$, 5.6; NaCl, 7.8; Na_2SO_4, 3.0; $CaCl_2$, 2.5; and $MgSO_4$, 2.5.

The total ionic content, anions or cations, of this solution is 21.4 meq liter^{-1} as compared with 4.1 for our regular synthetic freshwater, but its calcium content is only 25% higher (2.5 vs 2.0 meq liter^{-1}).

Simple electrolyte solutions were used to study electrolyte influences on the interaction of poliovirus with selected substrates. Solutions of simple salts in 1.0 mM $NaHCO_3$ were intially adjusted to pH 7.0. (The background electrolyte was chosen to ensure the media had some buffering capacity and to allow pH measurements to be made more reliably on the small volumes of suspension available; it was also present in similar concentration to that in the synthetic freshwater.) The final concentrations of the salts employed were: NaCl, 3.0, 10, and 30 mM; $CaCl_2$, 0.1, 0.3, 1.0, and 3.0 mM; $Al_2(SO_4)_3$, 0.015, 0.045, and 0.15 mM [5, 15 and 50 ppm as $Al_2(SO_4)_3$]; and Na_3PO_4, 0.1 mM.

Sewage effluents were collected from the sewage treatment plant in Ravena, Albany County, New York, which uses settling followed by activated sludge treatment. Both primary (settled) and secondary effluent were clarified by centrifugation at 5,000 rpm for 15 min. Primary effluent was also centrifuged at 27,000 *g* for 10 min, sterilized by passing it through a 0.22-μm membrane filter and stored at 4°C.

Coating of Sands with Bacterial Exopolymer

Ottawa and beach sands were steam sterilized twice for 15 min on 2 separate days. Samples (2 g) were placed in sterile 500-ml Erlenmeyer flasks containing 200 ml of either primary effluent clarified by centrifuging, or a culture of *Zoogloea ramigera* (ATCC 19544) which had been grown for 3–4 days at room temperature in a medium of the following composition: casitone, 0.5 g; glycerol, 0.5 g; Na-lactate, 0.05 g; yeast extract, 0.1 g per liter of clarified secondary effluent. After the flasks had stood overnight at room temperature, sufficient amounts of their contents were transferred aseptically to sterile 10-ml syringes, plugged at the bottom with glass wool to form soil columns approximately 1.5 cm high.

Filter-sterilized primary effluent, or *Zoogloea* medium were percolated at room temperature through the columns dropwise at a rate of about 100 ml day^{-1}. After about 40 days the effectiveness of the procedure was evaluated microscopically following staining.

As a control, the substrates were also briefly treated with a synthetic sewage solution containing, per liter, nutrient broth, 750 mg; $(NH_4)_2SO_4$, 225 mg; glucose, 750 mg; NaCl, 225 mg; KCl, 52.5 mg; $NaHCO_3$, 1,260 mg; and NaH_2PO_4, 187 mg. The solution was sterilized by autoclaving. Sand substrates (50 g) were shaken gently with 50 ml of the synthetic sewage solution for 10 min, and free liquid was removed. The sand was air-dried for 30 min at 22°C and then used for adsorption studies.

Bacteria were stained by the procedure described by Daley and Hobbie [5]. Acridine orange (0.1% in 100 ml distilled water) or alcian blue (0.3% in 100 ml 50% glacial acetic acid) were applied to the microscopic slide with the soil particles under a cover glass. After 20 min excess dye was removed by drawing distilled water under the cover glass with filter paper. The sample was examined under the microscope. The acridine orange stain was observed in a fluorescence microscope. The dye stains bacterial cells. On a coated particle, either a monolayer of cells separated by unstained exopolymer was seen, or the particle was covered with microcolonies. Alcian blue (in bright field illumination) shows the exopolymer either as a thin blue layer which was apparent particularly around the periphery of the sand particle, or as blue areas in face view representing polymer buildup in and around bacterial microcolonies.

Adsorption Experiments

Experiments were carried out as described previously [13]. Essentially, a 100-μl sample of the suspending medium containing 10^8–10^9 pfu and 10^3–10^4 cpm of virus was added to a slurry of 500 mg of substrate in 1.9 ml of the solution in a 50-ml Nalgene (no. 3119-0050) Oak Ridge-type polypropylene centrifuge bottle. The containers were shaken at 300 rpm on a gyratory shaker for 1 hour at 4°C followed by separation of substrate and suspending medium at 12,000 *g*. A temperature of 4° was chosen because it is closer to the temperature of many aquifers than room temperature and because it minimizes infectivity loss during incubation even with the more stable

Table 3. Poliovirus adsorption to 5 substrates: comparison of primary effluent, secondary effluent, and synthetic freshwater as suspending media

Substrate	Synthetic freshwater			Primary effluent			Secondary effluent		
	Final pH[a]	Log removal[b]		Final pH[a]	Log removal[b]		Final pH[a]	Log removal[b]	
		Inf.[c]	Rad.[d]		Inf.[c]	Rad.[d]		Inf.[c]	Rad.[d]
Ottawa sand	8.0	0.05	0.39	8.2	0.08	0.30	8.2	0.00	0.38
Riverhead sandy loam B-31	6.8	1.77	1.66	7.1	2.77	1.68	7.0	1.72	1.40
Muck	6.7	1.47	1.80	6.6	1.89	2.01	6.5	1.82	2.00
Genesee silt	7.4	2.42	2.11	7.5	2.40	1.82	7.4	2.72	2.09
Montmorillonite	8.0	1.16	1.10	8.4	1.89	1.54	8.3	1.72	1.70
Control	8.1	0.08	0.21	8.1	0.04	0.15	8.1	0.05	0.22

[a] The initial pH values of the synthetic freshwater, primary effluent, and secondary effluent were 8.0, 8.1, and 8.2, respectively, and were not adjusted before addition of the suspensions to the sampling container
[b] The values represent averages of duplicate samples in terms of percent virus adsorbed, then converted to log removal
[c] Infectivity
[d] Radioactivity

Table 4. Effect of suspending medium on echovirus 1 adsorption

Substrate	Log virus removal[a]			
	Synthetic freshwater		Secondary effluent	
	Inf.[b]	Rad.[c]	Inf.[b]	Rad.[c]
Ottawa sand	0.07	0.07	0.07	0.06
Montmorillonite	0.46	0.35	0.30	0.34
Genesee silt	1.47	1.66	1.40	1.39
Muck	1.43	1.85	1.47	1.72

[a] Values are based on the analysis of dilute stock suspensions. Analyses were in duplicate, percent recoveries were averaged before conversion to log removal
[b] Infectivity
[c] Radioactivity

viruses. Previous studies had shown that poliovirus adsorption at 4° reaches a plateau within 1 hour [13]. The pH of the suspending media was measured, and samples removed in duplicate or triplicate for infectivity and radioactivity measurements.

The amount of virus adsorbed was calculated as the difference between the amount recovered from suitable control samples and the amount found in the supernatant of samples containing substrate. The variability between duplicates was typically 10% and 15% of the mean for infectivity and radioactivity, respectively. Virus adsorption is expressed as log removal, 90% adsorption giving a value of 1.0, 99% as 2.0, etc.

In the tables, adsorption is described as loss of infectivity (as pfu) from the suspending medium and loss of radiolabeled virus (as cpm). Every preparation of purified virions contained a small amount (<0.1%–2.0%) of radiolabeled nonvirion protein. These products most likely represent virus-specific nonstructural proteins or the precursor of capsid protein which copurified with virions. By comparing the loss of virus infectivity with the removal of radiolabeled virions from the medium, it was possible to determine whether a significant proportion of the virions had eluted

from the substrate and were present in the medium as noninfectious particles. However, such a comparison is valid only if the amount of radiolabeled material recovered in the medium is greater than the amount of possible contaminating nonvirion radiolabeled protein (<2%). Conversely when a high level of loss of infectivity was detected and when the loss of radiolabel was also at least 95%, the infectivity data were the more meaningful and serve as the basis for interpretation.

Results

Comparison of Sewage Effluents with Synthetic Freshwater as Suspending Medium

Since virus adsorption is affected by organic solutes in the suspending medium, we performed initial experiments in which the synthetic freshwater was replaced by secondary or clarified primary effluents from a biological treatment plant. These experiments were carried out with poliovirus and echovirus 1. Table 3 shows results of an experiment with poliovirus and five substrates. There was no noticeable difference in the adsorption of poliovirus from the 3 media, except that the adsorption of virus to montmorillonite improved in the treated sewage. The slight difference in pH does not explain this, as at a pH value of 8 the ability of this mineral to adsorb poliovirus decreases with increasing pH values [18]. It is also known that the ability of montmorillonite to bind and exchange cations greatly affects its adsorptive properties for virus [11]. The adsorption of poliovirus to Riverhead sandy loam has shown great and random variability in other experiments. Therefore the effect of primary effluent seen in the results of Table 3 may not be significant.

Similar results were obtained with echovirus 1 (Table 4). Adsorption by montmorillonite in terms of infectivity was somewhat lower with the secondary effluent.

The wastewater used in these experiments had a calcium content of 3 meq liter^{-1}, 50% higher than in our synthetic freshwater, and a total dissolved solids content of 450 mg liter^{-1}, 25% higher. Both higher calcium content and increased ionic strength tend to promote virus adsorption. It appears that the effects of organic matter in the effluents and of different electrolyte contents were not large and of similar magnitude.

Bacterial Overgrowth

If primary wastewater effluent trickles over a clean glass surface, bacteria contained in the effluent may begin to colonize the surface within a few hours [22]. Subsequently they form either microcolonies or monolayers of cells encased in bacterial exopolymer. The rapidity with which overgrowth occurs depends on the nutrient content of the solution and other factors. In our experiments, growth was slow and therefore the substrates were treated for several weeks.

Of the three substrates used, Ottawa sand was more readily overgrown than the Lake Ontario beach sand and magnetite sand, and *Zoogloea* overgrowth was more extensive, covering 85% of the surface of the Ottawa sand and 25%

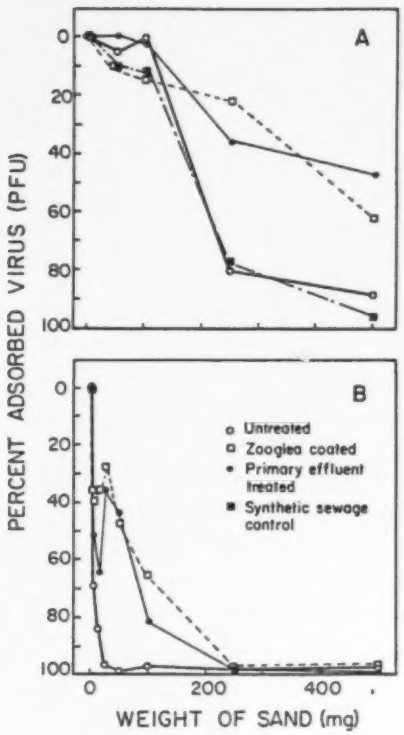

Fig. 1. (a) Effects of bacterial coatings on interaction of poliovirus with Ottawa sand. (b) Effects of bacterial coatings on interaction of poliovirus with beach sand. (Synthetic sewage control not run.)

of the other sands. The smaller grains of the two latter substrates were also seen adhering to or enclosed in floc-shaped microcolonies of bacteria, i.e., mixed aggregates. Approximately 25% of the beach sand (0.05–0.1 mm diameter particles) was associated with *Zoogloea* aggregates. Magnetite sand (0.15–0.25 mm diameter particles) was enclosed in the aggregates to a lesser degree with a greater portion of the surfaces colonized by *Zoogloea*. Alcian blue staining confirmed the presence of exopolymer on the surface of the sand grains beyond the microcolonies. The overgrowth by the bacteria from primary effluent did not stain with alcian blue.

Poliovirus Adsorption. Treatments of Ottawa sand and Lake Ontario beach sand included overgrowth with *Zoogloea*, overgrowth with indigenous bacteria from primary effluent, and treatment of the substrates with sterile synthetic sewage. With both substrates, the treatments with bacteria significantly reduced virus adsorption (Figs. 1a and b). Treatment with synthetic sewage alone, however, had very little effect on virus adsorption.

The greater surface area of beach sand as compared with Ottawa sand (0.73 m^2/g vs 0.01 m^2/g) may account for the more pronounced effect with beach sand at low substrate concentrations and for the complete adsorption by the higher concentrations of uncoated beach sand.

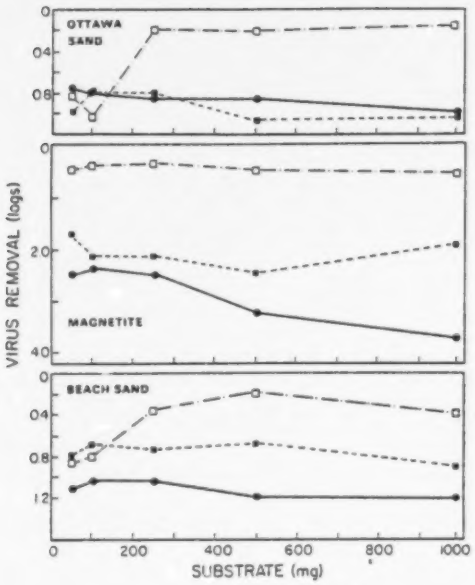

Fig. 2. Effect of bacterial overgrowth on reovirus adsorption to sand. Overgrowth with *Zoogloea ramigera* (□) and bacteria indigenous to primary effluent (■) on *Ottawa sand, magnetite sand,* and Lake Ontario *beach sand.* Untreated substrate served as control (●). Amount of substrate varied from 50 to 1,000 mg/2 ml. Virus concentrations were 2.5×10^8.

A Long Island soil (Riverhead Sandy Loam III, C-1 horizon) was either treated with secondary effluent or overgrown with *Zoogloea* as described before. When 50–500 mg of the treated soils was added to a poliovirus suspension, all of the virus was recovered, whereas the same amount of uncoated material retained between 30% and 70% of the virus (data not shown).

Reovirus Adsorption. The effects of bacterial coatings on reovirus adsorption are shown in Fig. 2. *Zoogloea* overgrowth reduced reovirus adsorption more than coating produced with bacteria from primary effluent. Adsorption of reovirus to magnetite sand, previously shown to be greater than 99.8% of 10^8 pfu [14] was reduced to approximately 60% by coating with *Zoogloea* (Fig. 2). Only a small reduction in adsorption was observed with beach sand (Fig. 2). For all three *Zoogloea*-coated substrates (Ottawa sand, magnetite and beach sand), the specific infectivity of virus remaining in suspension increased, suggesting greater preservation of virus infectivity. A substantial amount of *Zoogloea*-coated Ottawa sand was required to compete effectively with adsorption to the surface of the container (Fig. 2). *Zoogloea*-coated magnetite sand and beach sand showed this loss at much lower concentrations.

The Effect of Low-Temperature Ashing and Ignition of Substrates

Natural soil materials almost always contain organic material or have been in contact with water containing organic matter. Low-temperature ashing (LTA) oxidizes these materials without significant chemical alteration or physical

disruption of the material. In this manner, mineral surfaces are freed of organic matter. In our studies, both LTA and ignition at 550 ± 50°C were used to remove organic material. The influence of LTA on poliovirus adsorption was measured with six substrates. The adsorption of poliovirus improved upon LTA with all minerals except the Genesee silt (Table 1). The ashed Genesee silt adsorbs less of the cationic polyelectrolyte, polydiallyldimethylammonium chloride (PDADMA). In general, PDADMA adsorption is inversely correlated with poliovirus adsorption [13]. The explanation for this unexpected response of silt is not known. After ignition, the minerals are materially altered and effects on virus adsorption are less predictable.

Virus Adsorption with Increasing Soil Depth

In wastewater recharge beds, one can expect to find coatings from bacterial growth and adsorbed organic materials on the topsoil particles. We obtained soil samples for direct microscopic analysis from a recharge bed of the Lake George, N.Y., wastewater treatment plant. At Lake George, recharge beds have been in use for 35 years. The effluent is from trickling filters. The surface of the beds is occasionally raked or graded. Our samples were taken in September 1977 at 0–100 cm depth. Microscopic examination after staining with acridine orange and alcian blue showed complete colonization with bacteria only in a 5-mm-thick surface layer. The surface of the soil particles was covered with microcolonies consisting of many-layered bacterial growth. At 15 cm depth, sand grains were covered only partially with microcolonies, and at 90–100 cm the sand was clean or almost so. Very few microcolonies were seen, and some growth was seen covering opaque debris which presumably was organic detritus covered with bacteria.

The soils which were included in the virus adsorption experiments were an acid outwash soil typical of Long Island, N.Y., representative of future wastewater recharge sites, and the Acuff loam from Lubbock, Texas, which had been subject to irrigation with primary effluent for 40 years, together with untreated soil from an adjacent plot. At both sites, soil alkalinity increased and organic content decreased with depth. The surface areas of the material from both sites increased with depth; this is consistent with the decreasing proportion of coarse sand on progression down the soil profile. A higher content of clay minerals (fine particles), which can bind water too firmly to be readily removed at 110°C and atmospheric pressure, may be responsible for the greater weight loss of the deepest soils upon more thorough desiccation as in the low-temperature asher or upon ignition. In the case of the Acuff samples, irrigation had resulted in the buildup of organic matter in the topsoil which tends to compete with or reduce virus adsorption and possibly also in a saturation of mineral surfaces with virus. The question then arose whether such soil may have lost some of its capacity for virus adsorption.

Relevant physical and chemical properties of the substrates from several soil series were shown in Table 2; virus adsorption data for poliovirus, echoviruses 1 and 7, and Coxsackievirus are shown in Table 5.

In every instance, the adsorption of virus increased with soil depth (Table

Table 5. Soil series: virus adsorption

Substrate (no., type)	Depth (cm)	Poliovirus 2 Log removal Inf.[b]	Poliovirus 2 Log removal Rad.[c]	Poliovirus 2 Final pH	Echovirus 1 Log removal Inf.[b]	Echovirus 1 Log removal Rad.[c]	Echovirus 1 Final pH	Echovirus 7 Log removal Inf.[b,d]	Echovirus 7 Log removal Rad.[c,e]	Coxsackievirus B5 Log removal Inf.[b]	Coxsackievirus B5 Log removal Rad.[c]
Genesee silt loam											
24	15–30	0.60	1.14		0.22	0.21		0.95	0.71	0.72	0.57
29	30–60	1.68	2.12		1.69	1.54		1.18	0.79	2.52	2.15
Riverhead sandy loam											
52 (B21)	2–12	0.89		5.3	1.52	1.75	4.9				
53 (B22)	12–40	1.28		5.5	2.14	1.20	5.0				
54 (B31)	40–60	1.55		5.6	2.24	1.23	5.1	0.79	0.42		
55 (B32)	60–90	1.52		6.3	2.49	2.44	6.1				
56a (III,C1)	90–150	2.15		6.9	2.32	1.31	6.1				
56 (III,C2)	150–180	2.63		7.4	1.33	1.14	6.5				
Acuff loam, irrigation field											
101	0–15	1.42	1.27		0.50	0.54	6.8	0.30	0.18	0.82	0.30
102	15–30	2.30	1.47		0.65	0.55	7.0	0.61	0.54	1.00	0.47
103	30–60	3.10	1.72		1.26	0.62	7.3	1.50	0.64	1.40	0.68
Acuff loam, control field											
104	0–15	1.62	1.77		0.56	0.71	7.5	0.89	0.55	0.96	0.51
105	15–30	2.64	1.60		1.02	1.02	7.6	0.57	0.67	1.40	0.48
106	30–60	2.92	1.70		2.00	2.00	7.8	1.08	0.84	1.40	0.92

[a] Regular synthetic freshwater was used in all experiments
[b] Infectivity
[c] Radioactivity
[d] Log removal relative to the least adsorbing standard substrate (no. 30, muck) (Moore et al. 1981[11])
[e] Log removal relative to the least adsorbing standard substrate (no. 2, montmorillonite) (Moore et al. 1981[11]). Other removals are relative to a substrate-free control

5). This behavior paralleled the decrease in organic matter with depth as reflected in the measurements of organic carbon and of weight loss upon low-temperature ashing or ignition at 550°C (Table 2). The Acuff loam control soils did not exhibit a decrease in organic content with depth, but nevertheless they showed a strong increase in virus adsorption with depth.

Adsorption of the cationic polyelectrolyte PDADMA decreased with depth in all soils (Table 2), whereas other variables such as iron, aluminum, or calcium did not change with depth. This indicates a change of charge development with depth independent of changes in organic content.

In the Acuff loam which received secondary effluent, the buildup of organic matter in the topsoil was quite pronounced (Table 2). Virus adsorption was considerably decreased in that layer. Among all Acuff loams, however, the effect of soil depth was more pronounced than the effect of irrigation. It also appears that the virus binding capacity of the subsoils, even after 40 years of irrigation with wastewater, was not significantly exhausted. The virus infectivity losses in the two subsoils (30–60 cm) remain of comparable magnitude (Table 5).

Virus adsorption should improve with increased electrolyte content of the suspending medium. In our experiments with the 2 synthetic freshwater formulations, this effect was sometimes, but not consistently, seen (data not shown). The difference in the 2 synthetic freshwater formulations is mostly in the less active monovalent ions. The difference between air-dried and field-moist soils was not clear-cut either.

Both organic content and PDADMA adsorption were correlated with poliovirus adsorption in a sample of over 30 soils tested in our laboratory [13]. In the present sample, a correlation at the $P = 0.05$ level holds for organic carbon versus poliovirus and echovirus 1 adsorption ($r = -0.562$ and -0.654, 12 degrees of freedom), PDADMA adsorption versus poliovirus adsorption and echovirus 1 adsorption ($r = -0.672$), and weight loss on LTA versus poliovirus adsorption ($r = 0.684$). Equally conspicuous is the failure of echovirus-7 behavior in this sample to be predictable on the basis of either PDADMA adsorption or weight loss on LTA. The larger data set on the interactions of the viruses with over 40 substrates is being analyzed and the results will be published elsewhere (G. W. Fuhs, submitted for publication).

Discussion

The adsorption of virus to surfaces is governed by electrostatic and London-van der Waals forces. The contribution of both types of forces to the overall potential energy of interaction depends on the materials involved and the chemical composition of the aqueous medium. The details of these interactions were summarized earlier [9]. The strong effect of organic coatings on minerals in reducing virus adsorption points toward the importance of van der Waals attractive forces in the 3-component system: virus, solid surface, and water. They are greater for surfaces consisting of metals and metal oxides and water than for organic materials and water. Humic materials and bacterial exopolymer are highly hydrated organic materials and therefore exhibit low van der Waals

forces. The difference between the magnetite before or after overgrowth with bacteria is particularly spectacular, i.e., the amount of virus adsorbed is reduced more than a thousandfold. The contribution of charge to the interaction is relatively small as most of these organic materials carry only weak negative charges. The composition of *Zoogloea* exopolymer varies among strains [8, 19]. The strain used in this work, similar to the one described by Tezuka [19], produces a polysaccharide which contains glucosamine and very little uronic acid [7]. Humic matter carries carboxylic groups which dissociate in the pH range of 3–7 while its phenolic groups dissociate only at pH values of 8.5 and higher [23]. Humic materials are known to interfere with virus adsorption [1, 3].

Organic coatings on minerals are formed by adsorption and by bacterial overgrowth. In fact, practically all solid surfaces in the biosphere contain adsorbed organic microlayers [16]. The effect of these layers on virus adsorption cannot be readily predicted. In our experiments we see improvements of virus adsorption after removal of organic matter from substrates by low-temperature ashing. In agreement with these results, subsoils are consistently superior adsorbents than topsoils, and the adsorption in the latter can be impaired by the buildup of organic matter under the effects of wastewater irrigation. In many recharge beds, virus adsorption may still occur mostly in the top layers [6] of the soil because the equilibrium favors adsorption over desorption and provided that surface development is sufficient. Saturation of virus-binding sites as a result of prolonged exposure to virus-containing effluents is not evident.

A more accurate quantitative characterization of the binding of viruses to minerals and soils has been the goal of this and many other studies. In the course of our work we have come to believe that it is possible to characterize soils, in spite of their complexity, with respect to their most relevant properties either directly, e.g., by measurements of surface energy or electrophoretic mobility, or indirectly by measurements of organic oxide or polyelectrolyte content. There is, however, a need for a better characterization of the physical–chemical properties of the different viruses both in suspension under realistic environmental conditions and in the adsorbed state.

Acknowledgment. This work was supported by Grant Number R804743 from the US Environmental Protection Agency.

References

1. Bitton G, Masterson N, Gifford GE (1976) Effect of a secondary treated effluent on the movement of viruses through a cypress dome soil. J Environ Qual 5:370–375
2. Bitton G, Pancorbo O, Gifford GE (1976) Factors affecting the adsorption of poliovirus to magnetite in water and wastewater. Wat Res 10:973–980
3. Bixby RL, O'Brien DJ (1979) Influence of fulvic acid on bacteriophage adsorption and complexation in soil. Appl Environ Microbiol 38:840–845
4. Burge WD, Enkiri NK (1978) Virus adsorption by five soils. J Environ Qual 7:73–75
5. Daley RJ, Hobbie JE (1975) Direct counts of aquatic bacteria by a modified epifluorescence technique. Limnol Oceanogr 20:875–881
6. Drewry WA, Eliassen R (1964) Virus movement in groundwater. J Wat Pollut Control Fed 40:R257–R271

7. Farrah SR, Unz RF (1976) Isolation of exocellular polymer for Zoogloea Strains MP6 and 106 and from activated sludge. Appl Environ Microbiol 32:33–37
8. Friedman BA, Dugan PR, Pfister RM, Remsen CC (1968) Fine structure and composition of the zoogloeal matrix surrounding *Zoogloea ramigera*. J Bacteriol 96:2144–2153
9. Fuhs GW, Taylor DH (1982) Virus uptake by minerals and soils. In: Middlebrooks EJ (ed) Water reuse. Ann Arbor: Ann Arbor Science, Ann Arbor, Michigan, pp 603–639
10. Hinesly TD, Thomas RL, Stevens RG (1978) Environmental changes from long-term land applications of municipal effluents. EPA Technical Report, EPA/430/9-78/003 (*or* EPA/MCD-26)
11. Lipson SM, Stotzky G (1983) Adsorption of reovirus to clay minerals: effect of cation exchange capacity, cation saturation, and surface area. Appl Environ Microbiol 46:673–682
12. Lo SH, Sproul OJ (1977) Polio-virus adsorption from water onto silicate minerals. Wat Res 11:653–658
13. Moore RS, Taylor DH, Sturman LS, Reddy MM, Fuhs GW (1981) Poliovirus adsorption by 34 minerals and soils. Appl Environ Microbiol 42:963–975
14. Moore RS, Taylor DH, Reddy MM, Sturman LS (1982) Adsorption of reovirus 3 by minerals and soils. Appl Environ Microbiol 44:852–859
15. Oza PP, Chaudhuri M (1975) Removal of viruses from water by sorption on coal. Wat Res 9:707–712
16. Renn CE (1964) The bacteriology of interfaces: principles and applications in aquatic microbiology. In: Heukelekian H, Dondero NC (eds) Proceedings of the 1963 Rudolfs Research Conference at Rutgers University. Wiley, New York, pp 193–201
17. Scheuerman PR, Bitton G, Overman AR, Gifford GE (1979) Transport of viruses through organic soils and sediments. J Environ Eng Div ASCE 105:629–640
18. Taylor DH, Moore RS, Sturmann LS (1981) Influence of pH and electrolyte composition on adsorption of poliovirus by soils and minerals. Appl Environ Microbiol 42:976–984
19. Tezuka YA (1978) *Zoogloea* bacterium with gelatinous mucopolysaccharide matrix. J Wat Pollut Control Fed 45:531–536
20. Tofflemire TJ, Chen M (1978) Evaluation of phosphate adsorption capacity and cation exchange and related data for 35 common soil series in New York State. Technical Report no. 45. New York State Department of Environmental Conservation, Bureau of Water Research, Albany, New York, 300 pp
21. US Department of Agriculture Soil Conservation Service (1972) Soil Series of the United States, Puerto Rico and the Virgin Islands: their taxonomic classification. US Dept Agric Soil Cons Service, Washington, DC
22. Ware GC, Loveless JE (1958) The construction of biological film in a percolating sewage filter. J Appl Bacteriol 21:308–312
23. Wilson DE, Kinney P (1976) Effects of polymeric charge variations on the proton-metal ion equilibria of humic materials. Limnol Oceanogr 22:281–289

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